



## Determination of Extended-Spectrum Beta-Lactamases Genes and Antibiotic Resistance Patterns in *Escherichia coli* Isolates from Healthy Cats

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### ABSTRACT

**Background:** This study was set to detect extended-spectrum beta-lactamases (ESBLs) producing *E. coli* isolates and the genes underlying their resistance in relation to phylogenetic background from fecal samples of healthy owned cats.

**Methods:** A total of 50 *E. coli* isolates were confirmed by standard bacteriological tests. The phylogenetic analyses of the isolates were carried out by combinations of three genetic markers *chuA*, *yjaA* and DNA fragment *TspE4.C2* by a triplex PCR method. The ESBL (*bla*<sub>CTXM</sub>, *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub>, *bla*<sub>OXA</sub>) encoding genes were detected. To identify ESBL producing phenotypes, all selected isolates were screened with a double disk synergy test including cefotaxime, cefotaxime with clavulanic acid, ceftazidime and ceftazidime with clavulanic acid.

**Results:** Results showed that *E. coli* isolates fell into four phylogenetic groups (A, D, B1 and B2) with prevalence of 78%, 4%, 8%, 10% and five phylogenetic subgroups including A0 (74%), A1 (4%), B1 (8%), B2-2 (6%), B2-3 (4%) and D1 (4%), respectively. Among all *E. coli* isolates, 4% were positive for *bla*<sub>SHV</sub>, *bla*<sub>CTX-M-15</sub> and *bla*<sub>OXA-1</sub> genes which distributed in B2-2, B2-3, A0 subgroups, respectively. According to antibiotic susceptibility test, 20 isolates were resistant which belonged to D (D1 phylogenetic subgroup) and A (A0 phylogenetic subgroup) groups.

**Conclusion:** The results showed that healthy cats could be considered as potential source for the dissemination of ESBL-encoding genes. Further investigations in companion animals and their owners are needed to clarify the importance of spreading of these zoonotic strains.

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## Introduction

*Escherichia coli* is one of the primary causes of urinary tract infections and pyometra, particularly in companion animals and drug resistant *E. coli* strains have been associated with urinary tract and other nosocomial infections in veterinary hospitals (1-3).

$\beta$ -Lactams are widely used in veterinary medicine to treat infections caused by *E. coli* in dogs and cats. This widespread use may have contributed to the substantial increase in the emergence of *E. coli* resistance to  $\beta$ -lactams in companion animals over the last several decades (4-6).

The emergence of  $\beta$ -lactam-resistant bacteria in companion animals and the transfer of resistant isolates to humans pose a potential serious risk to public health (7). Other bacteria from these animals with resistance to extended-spectrum cephalosporins (ESCs), such as ceftiofur and ceftriaxone, could be considered as emerging pathogens (8). Resistance to ESCs in *E. coli* is mediated primarily by production of class A extended-spectrum  $\beta$ -lactamases (ESBLs) which hydrolyze oxyimino-cephalosporins but are not active against cephamycins and carbapenems and can be inhibited by lactamase inhibitors (e.g., clavulanic acid) (9). Most ESBLs and  $\beta$ -lactamase inhibitor-resistant  $\beta$ -lactamases are derived from the classical TEM-1, TEM-2, and SHV-1 enzymes as the result of amino acid substitutions in their sequences (8). The plasmid-encoded CTX-M family members, which confer high levels of resistance to ESCs, have been reported to be found worldwide in *E. coli* strains isolated from human and other animal sources (4, 10-13). CTX-M  $\beta$ -lactamases exhibit greater activity against cefotaxime than ceftazidime (10). ESBLs in the clinical strains of *E. coli* isolated from humans and food-producing animals have been characterized in various studies (11, 12, 14, 15), but few studies have been performed on *E. coli* isolates from dogs or cats (4). *E. coli* strains from companion animals producing CTX-M-1, CTXM-15, CMY-2, and SHV-12 have been found in Europe (16, 17), whereas strains with CTX-M-14 were reported in Chile (18). Recently, CTX-M-14, CTX-M-15, and SHV-12 ESBLs

have been identified in canine and feline *E. coli* isolates associated with urinary tract infections (UTIs) in the United States (19, 20).

There are limited data about the diversity and prevalence of resistance to broad-spectrum  $\beta$ -lactams among Enterobacteriaceae isolated from companion animals in Iran. This study was set to detect extended-spectrum beta-lactamases (ESBLs) producing *E. coli* isolates and the genes underlying their resistance in relation to phylogenetic background from fecal samples of healthy owned cats.

## Materials & Methods

### *Sampling and bacteriological examination*

During November 2013 to June 2014 rectal swabs was gathered from 50 apparently healthy owned cats which referred to veterinary hospital of Shahid Bahonar university of Kerman for checkup and vaccination. In the laboratory, samples were cultured on MacConkey agar and EMB (Biolife Laboratories, Milano, Italy). Standard bacteriological methods were used to isolate and identify the *E. coli* strains. The isolates were confirmed to be *E. coli* by using biochemical API 20E identification system (BioMérieux, Marcy l'Étoile, France). The culture of each *E. coli* isolate was stored in Luria-Bertani broth (Invitrogen, Paisley, Scotland) with 30% sterile glycerol at  $-20^{\circ}\text{C}$ .

### *Antibiotic Susceptibility Testing*

To identify ESBL producing phenotypes, all selected isolates were screened with a double disk synergy test including cefotaxime (30  $\mu\text{g}$ ), cefotaxime with clavulanic acid (30/10  $\mu\text{g}$ ), ceftazidime (30  $\mu\text{g}$ ), ceftazidime with clavulanic acid (30/10  $\mu\text{g}$ ) [Dickinson and Company, Breda, Netherlands]. Results were interpreted according to CLSI guidelines (CLSI, 2010).

## Reference Strains

In this study, *E. coli* ECOR62 for (*chu A*<sup>+</sup>, *yja A*<sup>+</sup> and *Tsp EA.C2*<sup>+</sup>), *E. coli* (ATCC 35218) and *Kelebsiella pneumoniae* (ATCC 700603) were used as positive controls for ESBL genes determination. *E. coli* strain MG1655 and *E. coli* ATCC 25922 were used as a negative control. All the reference strains were from the bacterial collection of Microbiology Department of Ecole Nationale Veterinaire Toulouse, France.

## Phylotype and ESBLs genes Determination by PCR Assay

DNA of *E. coli* isolates and reference strains was extracted by lysis method (21). The phylogenetic analyses of the isolates were carried out by combinations of three genetic markers *chu A*, *yja A* and DNA fragment *Tsp EA.C2* by a triplex PCR method (22). The ESBL (*blaCTX-M*, *blaTEM*, *blaSHV*, *blaOXA*) encoding genes were detected by PCR as previously described (23). The primers used for amplification of the ESBLs genes and phylogenetic groups are presented in table 1, 2.

## Results

PCR assays indicated that *E. coli* isolates fell into four phylogenetic groups (A, D, B1 and B2), with prevalence of 78% (39 isolates), 4% (2 isolates), 8% (4 isolates), and 10% (5 isolates), respectively. Further analysis of PCR phylotyping showed that such isolates fell into five phylogenetic subgroups including A0 (74 %), A1 (4 %), B1 (8 %), B2-2 (6 %), B2-3 (4 %), and D1 (4 %). Among 50 *E. coli* isolates, 2 (4%) were positive for *bla SHV*, 2 (4 %) for *blaCTX-M-15* and 2 (4 %) for *blaOXA-1* gene which distributed in three phylogenetic subgroups as follow: B2-2, B2-3, A0. According to antibiotic susceptibility test, 20 (10 %) isolates were resistance against at least one antibiotic. Phylotyping of 20 antibiotic resistant isolates showed that these isolates belonged to D (D1 phylogenetic subgroup) and A (A0 phylogenetic subgroup) groups.

**Table 1.** The primers used for amplification of phylogenetic groups.

Gene	Primer sequence (5'-3')	Product size (bp)	Reference
<i>yjaA</i>	TGA AGT GTC AGG AGA CGC TG	211 bp	
	ATG GAG AAT GCG TTC CTC AAC		
<i>TspEA.C2</i>	GAG TAA TGT CGG GGC ATT CA	152 bp	
	CGC GCC AAC AAA GTA TTA CG		
<i>chuA</i>	GAC GAA CCA ACG GTC AGG AT	279 bp	
	TGC CGC CAG TAC CAA AGA CA		

**Table 2.** The primers used for amplification of the ESBLs genes.

Gene	Primer sequence (5'-3')	Product size (bp)	Reference
<i>TEM</i>	ATAAA ATTCTTGAA GACGAAA	1080 bp	Sharma et al (2010)
	GACAGTTACCAATGCTTAATC		
<i>SHV</i>	GGGTAATT CTTATTGTGCGC	928 bp	Sharma et al (2010)
	TTAGCGTT GCCAGTGCTC		
<i>CTX-M-15</i>	CGCTTTGGATGTGCAG	550 bp	Massai et al (2006)
	ACCGGATATCGTTGGT		
<i>OXA</i>	TCAACTTTCAGATCGCA	609 bp	Colom et al (2003)
	GTGTGTTTGAATGGTGA		

## Discussion

Examination of antimicrobial susceptibility patterns in commensal *E. coli* allows for comparison of resistance across different animal systems and antimicrobial use practices (24). Most companion animal antimicrobial resistance studies have been derived from diagnostic laboratory submissions, zoonotic transmission case reports, or non-representative populations (17). Data derived from these sources are useful, but they provide, at best, only partial insight into the prevalence of antimicrobial resistance in companion animals, so epidemiological studies in this field must be performed to clarify the obscurities.

Our study shows that ESBL-producing *E. coli* strains can be isolated from companion animals and most ESBL-producing *E. coli* strains exhibited the multidrug resistance (MDR) phenotype.

In a study on healthy cats (n = 36) and dogs (n = 39), two *E. coli* isolates, both from the same dog, showed reduced susceptibility to cefotaxime indicating a prevalence of 2.6%

whereas no isolate was resistant to cefotaxime in cats (17). Murphy *et al.* (25) reported that in two regions in southern Ontario 0% of both cats (n = 39) and dogs (n = 188) were positive for *E. coli* with reduced susceptibility to cefotaxime. Gandolfi-Decristophoris *et al.* (26) showed that in Switzerland, the fecal swabs taken from the healthy cats (n= 202) and dogs (n= 174) at nursing homes and at veterinary clinics were positive for Enterobacteriaceae with reduced susceptibility for the 3rd generation cephalosporins for examined dogs (17%) and cats (12%). In addition, 2.9% of the dogs and 2% of the cats examined were positive for ESBL genes. These results are in agreement with our findings. In the previous studies, *E. coli* isolates positive for *bla* *oxa-1* were mainly belonged to B2 phylo-group which is in accordance with the current study (6).

Surprisingly, in the present study, there was no conformity between the results of genotypic and phenotypic antibiotic resistance. Although the  $\beta$ -lactamase production is the most common mechanism of resistance, multiple mechanisms of resistance may work together in producing resistance to a given class of antibiotics. For example, the entry of any beta-lactam antibiotic into the bacterial cell is via outer membrane proteins, which function as receptors through which the antibiotics pass. These proteins may have lost or changed, contributing to decreased entry of the antibiotic and reduced antimicrobial activity. On the other hand, in the three strains which possess *bla* *CTXM*, *bla* *SHV*, *bla* *oxa* genes but didn't show resistance pattern phenotypically were not expressed.

It can be concluded that in recent years number of reports from companion animals which infected or colonized with clinically and epidemiologically important multiple-drug resistant organisms were obviously increased (27, 28).

The sample size in the present study (50 animals) was relatively small. Therefore extrapolation of the results toward the general population of cats should be done with great care. The comparisons made to other studies are regarded appropriate since these studies included sample sets of more or less comparable size (24, 25, 29 ) Our

results suggest the need for better long-term surveillance of ESBLs among companion animal isolates in parallel with human and other animal isolates.

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### Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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